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Research report

Differences in the performance of NK1R−/− ('knockout') and wildtype mice in the 5-Choice Continuous Performance Test

Ashley J. Porter[a,1], Katharine Pilldige[a,1], S. Clare Stanford[a,*], Jared W. Young[b,c]

[a] Department of Neuroscience, Physiology and Pharmacology, University College London, Gower Street, London WC1E 6BT, UK
[b] Department of Psychiatry, University of California San Diego, La Jolla, CA, USA
[c] Research Service, VA San Diego Healthcare System, San Diego, CA, USA

HIGHLIGHTS

- We compared the behaviour of NK1R−/− mice and wildtypes in the 5-Choice Continuous Performance Test.
- NK1R−/− mice did not express excess impulsivity (premature response or false alarms) in this test.
- NK1R−/− mice expressed excessive perseveration, which is common in ADHD.
- The findings point to a behavioural phenotype for ADHD patients with polymorphism of the *TACR1* gene.

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ABSTRACT

Mice lacking functional NK1 (substance P-preferring) receptors typically display excessive inattentiveness (*omission errors*) and impulsivity (*premature responses*) when compared with wildtypes in the 5-Choice Serial Reaction-Time Test (5-CSRTT). These abnormal behaviours are analogous to those seen in humans suffering from Attention Deficit Hyperactivity Disorder (ADHD). Here we used the 5-Choice Continuous-Performance Test (5C-CPT) to ascertain whether NK1R−/− mice also display excessive *false alarms* (an inappropriate response to a '*no-go*' signal), which is another form of impulsive behaviour. NK1R−/− mice completed more trials than wildtypes, confirming their ability to learn and carry out the task. At the start of Stage 1 of training, but not subsequently, they also scored more *premature responses* than wildtypes. When the mice were tested for the first time, neither *false alarms* nor *premature responses* was higher in NK1R−/− mice than wildtypes but, as in the 5-CSRTT, the latter behaviour was strongly dependent on time of day. NK1R−/− mice expressed excessive *perseveration* during all stages of the 5C-CPT. This behaviour is thought to reflect compulsive checking, which is common in ADHD patients. These findings point to differences in the 5-CSRTT and 5C-CPT protocols that could be important for distinguishing why the cognitive performance and response control of NK1R−/− mice differs from their wildtypes. The results further lead to the prediction that ADHD patients with polymorphism of the *TACR1* gene (the human equivalent of *Nk1r*) would express more *perseveration*, but not *false alarms*, in Continuous Performance Tests when compared with other groups of subjects.

1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a highly prevalent illness, which persists into adulthood in the majority of cases [1,2]. In childhood, patients suffer from hyperactivity, inattention and impulsivity, which contribute to difficulties at school and everyday life [3,4]. As adults, ADHD patients commonly experience co-morbid social, occupational and health problems [3,5].

A limited number of treatments for ADHD are available, but these were not targeted at the neural mechanisms underlying the disorder because these mechanisms have yet to be identified. Amongst the candidates thought to cause, or increase vulnerability to, ADHD is polymorphism(s) of the (human) *TACR1* gene [6,7], which is equivalent to the *Nk1* (substance P-preferring) receptor gene in rodents. Delineating a role for the *Nk1r* gene in behaviours relevant to ADHD could enable more targeted development of drug treatments for this disorder.

* Corresponding author.
E-mail address: c.stanford@ucl.ac.uk (S.C. Stanford).
[1] Contributed equally to the experiment.

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Mice with functional ablation of the *Nk1r* gene have been generated ('NK1R−/−') [8] and we have reported previously that the males are hyperactive in a range of environmental contexts: *e.g.* an activity meter [9]; a light/dark exploration-box [6,10,11]; and in their home-cage [12]. Also, when assessed for the first time in a widely-used test of sustained attention, the 5-Choice Serial Reaction-Time task (5-CSRTT) [13,14], NK1R−/− mice typically respond before stimuli appear (*premature response*, a form of motor impulsivity) and miss responding to stimuli (*omission error*, a measure of attention) [11,12,15,16]. These behavioural abnormalities are arguably analogous to the hyperactivity, impulsivity and inattention seen in ADHD patients.

The inattention and response disinhibition of ADHD patients, however, is more commonly quantified using procedures such as the Conner's Continuous Performance Test (CPT) and the Test of Variables of Attention (TOVA: [17–19]). Both the CPT and TOVA include 'target' ('*go*') trials, to which subjects should respond, and 'non-target' ('*no-go*') trials, to which they should withhold any response (*false alarms,* in the breach) [19–21]. In the latter case, these tests differ from the 5-CSRTT, which incorporates only ('*go*') targets that require responses. For this reason, a 5-Choice Continuous-Performance Test (5C-CPT), which includes both target and non-target trials, was developed to assess vigilance in mice [20–22], rats [23,24] and humans [22,25,26]. A preclinical study relevant to ADHD has reported that both methylphenidate and atomoxetine, which are licensed to treat ADHD, improve attention and reduced response disinhibition in rats that are performing poorly in the 5C-CPT [27].

Another important feature of the 5C-CPT is that, by including non-target stimuli, it enables quantification of impulsivity in the form of both *premature responses* (as in the 5-CSRTT) and *false alarm* responses (as in human CPTs). This differentiation is important because there is now extensive evidence that there are several aspects of impulsivity [28] and that different types of impulsivity recruit different neuronal networks [29,30]: *i.e.* these measures are neuro-mechanistically dissociable [21]. A further difference between the two tests is that a progressive decrement in vigilance develops when rodents are tested in the 5C-CPT [20,21,23], which is rarely seen in the 5-CSRTT.

Here, we further interrogated the putative association between the NK1 receptor and ADHD by comparing the performance of NK1R−/− mice and their wildtypes during training and testing in the 5C-CPT. We hypothesized that *Nk1r* 'knockout' mice would exhibit increased inattention and impulsivity (*premature responses* and *false alarms*), which are quantified in the 5C-CPT as lower *vigilance*.

2. Materials and methods

All procedures complied with the Animals (Scientific Procedures) Act (UK) [2010/63/EU] and had received local ethical approval at University College London.

2.1. Apparatus

The apparatus, described in detail elsewhere [15], was supplied by Med Associates (St. Albans, VT, USA) and was controlled by a Smart Ctrl Package 8IN/16OUT with an additional interface by MED-PC for Windows (Med Associates, St. Albans, VT, USA). The software was refined to incorporate a *no-go* signal (see: [20]).

2.2. Animals

All the mice were bred at University College London and housed in a facility at 21 ± 2 °C, 45 ± 5% humidity, with a 12/12 h light/dark cycle (lighting increased in steps from 07.00 to 08.00 h). We used twelve male wildtype mice (aged 6–7 weeks; weight: 30–34 g at the start of the study) and twelve male NK1R−/− mice of the same age-range (weight: 29–31 g). Inbred homozygous mice, rather than the (F2) offspring of heterozygous breeding pairs, were studied. This is because the incidence of *premature responses* in the 5-CSRTT (but not that of *omissions* or *hyperactivity* during the dark phase) depends on an interaction between a lack of *Nk1r* and breeding environment and is typically higher than their wildtypes only in inbred homozygotes [see: [12]]. The two genotypes shared the same background strain (129/Sv × C57BL/6J, crossed with outbred MF1 mice, many (more than 10) generations ago [8]). Wildtype mice were taken from two breeding pairs and NK1R−/− mice were taken from three breeding pairs and were group-housed as littermates (2–5 per cage). The home-cages incorporated environmental enrichment (cardboard tunnels and tissue for nesting material) and were cleaned twice weekly (bedding: 3Rs Bedding Pty., Ltd.). They were given free access to water throughout, but were fed a restricted diet (2018 Global Rodent Diet, Harlan) so as to maintain their body weight at 90% free-feeding weight. Every weekday, all the mice were weighed before training/testing in the 5C-CPT and fed between 16.00 and 17.00 h (after training/testing) with a quota of food determined by their body weight. At weekends, when there were no training or testing sessions, the mice were fed with 50% of their daily quota in the morning (between 09.00 and 11.00 h) and the remainder was given in the afternoon (between 16.00 and 18.00 h).

2.3. Training

At the start of the experiment, each mouse was assigned to one of four test chambers, counterbalancing for genotype, time of day (for training/testing) and home cage. This configuration was maintained throughout the experiment. Half the cohort was trained/tested in one of the morning sessions (three sessions were run between 10.00 and 12.00 h). The remainder were assigned to one of the afternoon sessions (three sessions were run between 13.00 and 15.00 h). Individual mice were trained/tested at the same time each day. All the behavioural data were captured and stored on-line. Each daily training session lasted for 120 trials or 30 min, whichever occurred first.

The animals carried out the training/test sessions with the house-light switched off (unlike the 5-CSRTT). After every correct trial, they were rewarded by delivery of an aliquot of sweetened milk (10 μL), which was available for 4 s. If the mice committed an *omission* error, commission error (*premature response* or *false alarm*), or incorrect response, the house-light was turned on for 5 s, as a 'punishment', during which time a new trial could not be initiated ('time out').

During Stage 1 of training, the animals experienced only '*go*' trials, which were delivered at a fixed interval (5 s; fixed 'ITI', as in the 5-CSRTT). In Stage 2, the *go* signal was delivered on a variable intertrial interval schedule (VITI: 3–7 s). Subsequent stages (3 and 4) incorporated *no-go,* as well as *go,* signals that were delivered with a VITI schedule: the ratio of *go:no-go* signals was 2:1 during Stage 3 and 5:1 during Stage 4. The mice graduated from one stage of training to the next when they had satisfied the performance criteria for a minimum of three consecutive days.

After reaching the performance criteria in Stage 4 ('baseline': Table 1), each animal was tested on the following Friday in an extended series of trials ('NI-1'). One difference between NI-1 and Stage 4 of training was that the number of trials was increased to 250 trials or 60 min, whichever was reached first. Another was that the ITIs were increased to 7–11 s. These were delivered in a random sequence, with the *go:no-go* signals remaining at a 5:1 ratio. As a consequence, the signal parameters in NI-1 in this 5C-CPT combined an increase in the latency of the ITIs (reduced event-rate)